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# NUTRITION OF THE CULTIVATED MUSHROOM

J. FRANKLIN STYER

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A THESIS

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA
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# NUTRITION OF THE CULTIVATED MUSHROOM

J. Franklin Styer

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The art of mushroom growing has been passed on from generation to generation without the development of exact methods. There is a great and costly lack of understanding of the biological processes which influence this culture. This situation must be charged to the fact that until the last few years the principles of spore germination and spawn production were poorly understood; and students who developed spore germination methods usually entered the spawn business without carrying on further investigations. Although the attitude of the spawn producers toward experimental work has been very helpful to the industry, they have in most cases lacked laboratory facilities for the kinds of work that were needed. Fortunately the development of the industry in America and Germany is leading to a concerted attack on the problems of mushroom growing, by specialists in the lines involved.

This study concerns the growth of the mycelium of the cultivated mushroom on simple media of known composition. The natural habitat of this mushroom is the sod of meadows and plains, but the medium used in mushroom growing is a partially decomposed preparation of horse manure. Chemical studies of this medium were begun by the writer along with this investigation, but the complexity of manures is such that the value of any such analyses is as yet uncertain. The record of growth on simple media throws more light on the nutrition of the organism and forms a more reliable basis for further work.

The literature on the subject of mushroom nutrition was reviewed in a preliminary paper (10). The outstanding work of Duggar (2) was the only one known to the writer in which a study of nutrition was the chief aim. Duggar's cultures were made upon filter paper dampened by nutrient solutions, and the physical nature of the media was supposed to resemble that of manure. His results with a series of carbonaceous materials indicated that the organism preferred starch as compared with sugars, and proteins above all other compounds. Salts of organic acids were apparently of no value. As sources of nitrogen the proteins were best, and growth was uniformly better with ammonium salts than with nitrates. Calcium hippurate seemed useful as a source of nitrogen.

The writer's first study (10) of this problem was carried out on the same plan as Duggar's. The ratio by weight of liquid to paper in Duggar's flasks was about 4 to 1, or 400 percent moisture, while the ratio in the writer's was 2 to 1, or 200 percent, and there was better growth by reason

of better aëration. The importance of aëration as a factor will be shown later in the present paper. The results of this series agreed in general with those of Duggar's, but it was found that the paper base alone could serve as a nutrient. It was also shown that while ammonium salts may be fair sources of nitrogen, the total nitrogen in that form in the medium must be small, about 0.1 molar. Complex organic compounds could be added safely in much larger amount. In both nitrogenous and carbonaceous series the more complex and less soluble nutrients gave the best results.

Careful examination of these results and comparison with Duggar's led the writer to suspect that the poor growth of the cultures upon soluble carbohydrates and nitrogenous compounds might have been due to the higher concentration of those media, rather than to their unsuitability as nutrients.

## Concentration of Media

In the preliminary study the solution of nutrient salts with which the paper was dampened had a concentration of 0.172 *M*. All cultures in which other soluble materials were added to the extent of 0.1 *M* or over, failed completely. Duggar's solution *A* was made up of salts totaling 0.16 *M*. He added to this solution other soluble materials; for example, 1½ percent dextrose, the concentration of which is 0.08 *M*. In both these series the salts were highly dissociated, further raising the concentration from the osmotic point of view.

A new series of cultures was made to ascertain the effect of concentration, with graded concentrations from 0.1 *M* to 0.3 *M*, and with more than one nutrient balance. It was found that the total concentration must be below 0.2 *M* for vigorous growth, and that the partial concentrations of individual nutrients may be varied without effect on this maximum point. Dissociation of salts, however, must be considered, and in a solution of 0.2 *M* concentration highly dissociated salts may be permitted up to 0.1 *M* only.

This result affords a good reason for the apparently greater value of the less soluble nutrients. The addition at the beginning of soluble nutrients, for example dextrose, might raise the osmotic effect of the medium to a point where the mycelium would not be able to grow at all. The insoluble materials, for example starch, could presumably be added in larger quantity, and be made available gradually by enzym action. This affords also a possible reason for the better growth obtained by Duggar in washed manure. It is a practice of spawn producers to wash the manure used for pure cultures. From the same considerations it would be reasonable to expect that the addition of soluble nutrients to manures used in mushroom growing would harm the spawn. This would depend upon the concentration of the manure solution and the time when the application is made. In Duggar's experiments the results of such additions were negative. Experiments upon manures and other media for mushroom growing, which are becoming numerous among the growers, should be carried out with careful consideration of this important factor.

## Moisture Content

The growth of the mycelium is indifferent in liquids. The species seems to be intensely aërobic, and too much moisture in fibrous media such as manure will invariably produce an adverse effect upon growth. The danger point in moisture content appears to be the degree of saturation at which free movement of air is cut off.

To determine this degree a number of test-tubes were tightly packed to a depth of 10 cm. with manure which had been washed with distilled water, and distilled water added so as to obtain a series with moisture content ranging from 125 percent to 250 percent of dry weight. The latter figure was the limit of moisture content so that aëration was cut off except through water. The tubes were inoculated at the surface of the medium, and the growth downward was measured. Growth was best at 125 percent, fell off very slowly to 200 percent, and at 225 percent dropped to less than half. With a total volume of manure of 25 cc., the air included at 125 percent moisture was 11 cc., and at 225 percent was reduced to about 3 cc. At saturation no downward growth occurred.

A second experiment in flasks with filter paper and the nutrient solution used in the preliminary investigation (10) gave similar results. This series covered a range from 50 percent to 350 percent moisture content. Growth failed below 100 percent, after a feeble start; it was good up to 233 percent, and then fell off sharply. Aëration was better in the flasks than in the tubes, having been actually cut off only at 300 percent. Aëration in a mushroom bed six inches deep should be good at 200 percent, if, as is usual, the surface is allowed to dry.

It is worthy of note that the nutrition experiments of Duggar discussed above seem to have been carried out with 25 cc. of solution added to each 6 gms. of paper, which would be a condition very unfavorable to growth. Many of his cultures grew only at the surface. The results would probably have been clearer if the moisture content had been better adjusted.

All the cultures affected by high moisture content developed excessive numbers of mycelial strands. These have recently been described at length by Hein (6), and were attributed by him to the stimulus of moist conditions. He stated the least number of strands were produced at a point between 45 percent and 80 percent moisture content; and although the impression was given that the best growth occurred at this point also, that was probably not his intention. There is a tendency to formation of strands in old cultures, and spawn producers are often forced to discard a strain which becomes "stringy." Certain organic nutrients cause strand growth as will later be shown. But if other conditions remain the same, the writer agrees with Hein that increases in moisture result in increases in the number and size of the strands.

Since the strands form profusely in the wetter media, and since they are also essential to fruiting through the translocation of food materials, a

connection between aëration or respiration and fruiting is suggested. The grower maintains his beds dry up to a certain point, and then induces the production of sporophores by watering. It seems probable that while the initial growth is favored by perfect aëration, fruiting is brought about by reduction of aëration, and the addition of moisture is a means of reducing it. Many other factors enter into crop development, but a study of respiration in this connection seems to the writer to have great possibilities.

## Silica Gel Medium

After the preliminary study brought out the fact that the cellulose of filter paper could support growth, and after agar was found to have inherent nutrient qualities, the writer turned to silica gel as an inert solid medium. It is an ideal medium for this type of work, for with surface growth aëration ceases to be a factor requiring consideration. The mycelium can also be observed with ease and comparisons can be made more exactly. The development of this medium has been described in a recent paper (11).

The medium was prepared by mixing two solutions; a potassium silicate solution 0.2 *M* with respect to $K_2O$, and an acid solution consisting of $H_3PO_4$, 0.135 *M*; $MgSO_4$, 0.015 *M*; $CaCl_2$, 0.006 *M*; and $FeSO_4$, trace. For the series of non-nitrogenous nutrients $NH_4NO_3$ was added to the acid solution in 0.15 *M* concentration. The alkaline solution was poured into the acid solution until a pH of 6.6 was reached, the optimum for this species according to Frear, Styer, and Haley (5), and water added until the volume was one and one-half times the volume of acid solution used. Thirty cc. of this mixture were poured into each plate and allowed to gel. The plates were dialysed in a dishpan full of tap water until about half the soluble materials were removed. The nutrient gel was then of the following composition: $MgSO_4$, 0.005 *M*; $CaCl_2$, 0.002 *M*; $FeSO_4$, trace; potassium phosphates, 0.045 *M*. The gel framework was a 1.2 percent content of $SiO_2$, hydrated. When $NH_4NO_3$ was present its concentration was 0.05 *M*.

After the silica gel plates had been dialysed, the substances whose nutritive value was to be tested were added in finely powdered form. The plates were then sterilized in the autoclave, and inoculated in the center with a small amount of a pure culture on manure. Within reasonable limits no difference in growth has been observed from different sized pieces of inoculum. During sterilization and subsequent standing the soluble substances diffused through the gel, while the others remained as even surface films. The amount of any insoluble material used was adjusted so that the film was not too thick to allow microscopic observation.

## Spore Germination

The cultures used were pure spore cultures, germinated and growing in washed fresh manure. A distinct strain of mushroom called "Snow-white" was used in all cultures. This strain originated in the beds of a Pennsyl-

vania grower; it may be recognized by its failure to develop pigments when grown in light. It is probably a strain of *Agaricus campestris*. No difficulty was met with in germinating the spores, after they had been collected under aseptic conditions. A brief description of the method follows.

Pieces of tissue with gills attached were removed aseptically from vigorous mushrooms before the rupture of the veil, and placed on match sticks in dry sterile Petri dishes. The sporophores naturally contain foreign organisms which may develop on the gills and cut surfaces of the pieces, and may contaminate the spore prints. This was prevented by permitting air to circulate under and around the pieces, which were cut between one and two centimeters square. Plates in which the tissue became damp or moldy were discarded, and the pieces were removed from all plates within 60 hours.

Small squares of sterile filter paper were drawn through the spore prints until heavily coated with spores, and then placed in tubes of sterile washed manure. The tubes were incubated at 25° C., and a mycelium was observed growing out from the spores in from six to ten days.

The quickness of germination and growth in these tubes is a matter of especial interest. The question arises whether the same principles are involved in this and the other known methods of stimulating germination of these spores.

The early work in this field, adequately reviewed by Miss Ferguson (4), failed to make known any dependable method of germination. Miss Ferguson found that the presence of mycelium or previously germinated spores of the same species served to hasten germination in hanging drops and to increase the number germinating to almost 100 percent. Duggar (2) found that this effect was not produced by sporophore tissue, but only by active mycelium. He obtained 10 percent germination in five days in a few solutions containing various acid phosphates. The reaction of these solutions was not discussed.

Richard and Olga Falck (3) advanced the hypothesis that one or more organic acids, produced by the fungous flora which normally precedes *Agaricus campestris* in outdoor manure beds, were the specific stimulants aiding germination. They obtained stimulation by the use of 0.25 percent succinic acid in malt and manure extracts and distilled water, the germination amounting to 50 percent in nine days. Other acids failed to stimulate germination, although they caused the same pH changes. Bechmann (1), in a series of tests with careful pH control, obtained the best germination, 100 percent in 19 days, at pH values between 5.0 and 6.0. At this reaction, attempts at stimulation with the aid of several enzym preparations and autolyzed tissues, including the tissue of *Agaricus campestris*, failed.

Bechmann considered the sugars in the malt extract used by him to have been the greatest factor in germination. Lambert (7) used a mixture of sucrose, maltose, and dextrose in agar and obtained plentiful germination.

Hein (6) obtained 75 percent germination in 14 days in distilled water alone.

These results lead to uncertainty as to what are the stimuli which cause germination. It is certain that the spores vary in their quickness to respond. Most of the investigators reported the germination of the spores in two groups several days apart. Miss Ferguson's explanation of this phenomenon is that the second group germinates only as a result of mycelial growth from the first. She cited the prompt germination of both these groups in drops containing mycelium from the beginning. The writer has obtained the same results in distilled water in test tubes.

This effect seems to have been obtained by the writer not only from mycelium, but from spores alone when massed. In making the stock cultures referred to above, the spores were in thick masses on the squares of filter paper, and an easily visible mycelium was produced within ten days. When the same approximate number of spores was distributed through the manure, or when the paper carried less spores, the mycelium appeared more slowly and sometimes did not appear at all.

It seems possible that carbon dioxid causes the germination of the more passive group of spores, either directly or through some influence on pH or oxidation-reduction potential. Apparent necessity for carbon dioxid in germination has been reported by Platz, Durrell, and Howe (8) for *Ustilago zeae*. Germination in a given time increased from 10 to over 50 percent by saturation of the solution with carbon dioxid. Unfortunately this was accompanied by a great change of pH and their cultures were not compared with cultures in which the pH was changed by other means. Rippel and Bortels (9) found that the removal of the carbon dioxid from the atmosphere in which the spores of *Aspergillus niger* were placed increased the time required for germination, and reduced the number germinating. When the few germinated spores produced a mycelium the rest germinated at once, a phenomenon quite like that observed by Miss Ferguson for *Agaricus campestris*. They added the following very interesting statement: "Now if germination did occur here and there over potassium hydroxid at so early a time, this was the case at only those places in which the spores lay together in thick heaps." The parallel between these cultures and those of the writer is striking.

If carbon dioxid is not a factor, some substance such as an acid or enzym, or some effect more difficult to detect, must emanate from the spores and mycelium to cause germination.

## Nutrition Experiments

The growth of the mycelium on the plates to which organic materials were added is recorded in table 1. The list of these materials was chosen so that it would represent all the classes of organic constituents of undecomposed and decomposing plant tissues as found in manure. Some types of soil organic matter were also included.

The diameter, density, and strand development of the mycelium were recorded after 18 days growth at 25° C. Growth continued in all the vigorous cultures until the plates were full, but the results were recorded at

TABLE 1. *Mycelial Growth of Agaricus campestris After 18 Days at 25° C. Upon Silica Gel Plates With a Variety of Organic Nutrients. Plates Contained $NH_4NO_3$, 0.05 M, Except Where the Organic Material Contained Available Nitrogen*

| Class | Material | Amount | Diameter (cms.) | Density | Strands |
|---|---|---|---|---|---|
| Acids | Succinic acid | 0.03 *M* | 3.0 | Sparse | Many |
| | Citric acid | 0.02 *M* | 0.0 | | |
| Sugars | Arabinose | 0.04 *M* | 3.5 | Medium | |
| | Xylose | 0.04 *M* | 4.5 | Dense | |
| | Dextrose | 0.04 *M* | 3.0 | " | |
| | " | 0.12 *M* | 3.5 | " | |
| | " | 0.20 *M* | 2.0 | Medium | |
| | Galactose | 0.04 *M* | 1.5 | Thin | Few |
| | Maltose | 0.02 *M* | 3.0 | Medium | |
| | " | 0.10 *M* | 4.5 | Dense | |
| | " | 0.10 *M* | 5.5 | " | |
| | Mannite | 0.04 *M* | 0.0 | | |
| Starch group | Starch from wheat | 0.25 gm. | 3.0 | Medium | Few |
| | Glycogen | 0.25 gm. | 3.0 | " | " |
| | Inulin | 0.25 gm. | 0.0 | | |
| Lecithin | —— —— | 0.25 gm. | 4.0 | Dense | Many |
| Cholesterol | —— —— | 0.25 gm. | 0.0 | | |
| Gums, pectins | Gum arabic | 0.25 gm. | 1.0 | Thin | |
| | Peach gum, from wound | 1.00 gm. | 6.5 | Dense | |
| | "Certo," commercial pectin | 2 cc. | 5.0 | Very dense | |
| Hemicelluloses | Xylan | 0.30 gm. | 2.5 | Dense | |
| | Agar | 0.55 gm. | 4.0 | Thin | |
| | Coconut, after ether extraction | 0.32 gm. | 2.0 | Dense | |
| | Wheat bran | 0.42 gm. | 5.5 | Very dense | Few |
| | Wheat straw | 0.32 gm. | 4.5 | Dense | |
| Cellulose | Shredded filter paper | 0.25 gm. | 1.0 | Thin | |
| | Ground cellulose | 0.25 gm. | 2.5 | " | |
| | " " | 0.25 gm. | 2.5 | " | |
| Ligno-complexes | *Liriodendron* wood | 0.25 gm. | 7.0 | Dense | |
| | " " | 0.36 gm. | 5.0 | " | |
| | German granulated peat moss, commercial "G.P.-M." | 0.20 gm. | 9.0 | Medium | Few |
| | Wood, cellulose removed by rot, 80 percent lignin | 0.25 gm. | 7.5 | Dense | |
| Humus | 2 percent $NH_4OH$ extract of rich soil | 0.25 gm. | 3.0 | Very thin | |
| | 2 percent $NH_4OH$ extract of rich soil | 0.15 gm. | 1.0 | " " | |
| | "Humus" from New Jersey deposits | 0.20 gm. | 2.5 | Dense | Few |
| Proteins, etc. | Nucleic acid from yeast | 0.30 gm. | 4.5 | Thin | |
| | Nucleoprotein from yeast | 0.10 gm. | 4.0 | Very thin | |
| | Peptone | 0.30 gm. | 3.5 | Dense | |
| | Gliadin | 0.30 gm. | 3.5 | Very dense | |
| | Glutenin | 0.20 gm. | 6.5 | Dense | |
| | Casein | 0.30 gm. | 4.5 | " | |
| | Albumin | 0.30 gm. | 5.0 | " | Many |
| Blank | No organic material | | 0.0 | | |

18 days because after that time strands began to appear in all the plates. The strands were recorded as "few" when appearing only in the center, and as "many" when extending within ½ cm. of the tips of the hyphae.

The pH of the gel was changed to 4.0 by the addition of succinic and citric acids. As this is far from the optimum pH, the results should be compared only with each other.

It is necessary to note that the materials containing hemicelluloses and lignin were quite impure chemically, and that their classification is only approximate. For example, wheat straw contains cellulose, gums, pectic substances, lignin, and hemicelluloses. Wheat bran was more correctly classified with the hemicelluloses. Woods of the class to which *Liriodendron* belongs contain about 50 percent cellulose, 20 percent pentosans, and 20 percent lignin. The granulated peat moss is much higher in lignin content. The black peaty soil from ancient bogs of New Jersey, sold under the name of "humus," may have been produced mainly from the lignin fraction of the original organic matter, but, like real humus, is quite different from lignin. For the selection and classification of these complex materials the writer has followed the principles of Waksman (12).

The growth of the mycelium upon plates with various nitrogen sources is recorded in table 2. It was necessary in the two series to supply every

TABLE 2. *Mycelial Growth of Agaricus campestris After 18 Days at 25° C. Upon Silica Gel Plates With a Variety of Nitrogen Sources. Plates Contained Dextrose, 0.035 M; Maltose, 0.035 M; and Starch, 0.1 gm., Except Where the Nitrogenous Compound Contained a Carbohydrate Group*

| Class | Material | Amount | Diameter | Density | Strands |
|---|---|---|---|---|---|
| Salts | $(NH)_2SO_4$ | 0.03 *M* | 2.5 | Medium | |
| | $NH_4NO_3$ | 0.05 *M* | 4.0 | Dense | |
| | $NaNO_3$ | 0.03 *M* | 1.5 | Thin | |
| Urine nitrogen | Urea | 0.05 *M* | 1.5 | Dense | |
| | Creatine | 0.05 *M* | 2.5 | " | |
| | Creatinine | 0.05 *M* | 3.0 | " | |
| Amino-acids | Glycine | 0.05 *M* | 3.5 | " | |
| | Glutamic acid | 0.05 *M* | 1.5 | Medium | |
| | Arginine carbonate | 0.03 *M* | 2.0 | Very dense | |
| | Leucine | 0.03 *M* | 4.0 | Dense | |
| Proteins, etc. | Nucleic Acid (Also in table 1) | 0.30 gm. | 4.5 | Thin | |
| | Nucleoprotein (Also in table 1) | 0.10 gm. | 4.0 | Very thin | |
| | Peptone (Also in table 1) | 0.30 gm. | 3.5 | Dense | |
| | Gliadin " " " " | 0.30 gm. | 3.5 | Very dense | |
| | Glutenin " " " " | 0.20 gm. | 6.5 | Dense | |
| | Casein " " " " | 0.30 gm. | 4.5 | " | |
| | Albumin " " " " | 0.30 gm. | 5.0 | " | Many |
| Blank | No nitrogen | | 4.0 | Sparse | |

culture with all nutrients except the one to be tested. This was done in the case of the organic series by adding ammonium nitrate whenever the organic nutrient did not contain nitrogen in a form already proved available.

In the nitrogen series, when the nitrogen source did not contain carbohydrate groups, dextrose and maltose were added, each in 0.035 molar concentration, along with about 0.1 gm. of starch. The compounds are grouped in the table according to complexity, with a separate group for nitrogen forms of urine.

The growth was dendritic and the mycelium was irregular in outline in the cultures on sugars. The peach gum and pectin cultures were quite different, spreading evenly and entirely in hyphal form, with many aërial hyphae. The aërial hyphae of the pectin plates presented a powdery appearance, due to great numbers of crystals on their surfaces. The mycelium changed most of the materials classed with the hemicelluloses into semi-liquid pastes. Xylan and fat-free coconut when thus liquefied practically submerged the mycelium, and may have interfered with its growth; the growth was dense, however, in advance of the liquefied zone. The growth on wheat bran, straw, and all the lignin-bearing materials was much the same as the typical dendritic growth on maltose.

The protein cultures resembled the xylan cultures in that the growth was dense, and accompanied by active digestion. The hyphae were straight, pointed directly outward, and were surprisingly equal in length; their tips were observed emerging from the liquefied protein mass unbranched and equidistant, like the teeth of a comb. This type of growth was quite different from the dendritic growth of the maltose cultures, and the enlargement into strands was much slower. Active leading growth in the maltose plates was focused into a few hyphae, and strands were built up from those hyphae after the plates became full. This was not the case in the protein plates.

The growth on nucleic acid and nucleoprotein was extremely thin, with long, unbranched hyphae. This effect was not noted in other plates. The mycelium had a silky appearance, with no strands.

The absence of nitrogen except in the inoculum resulted in the production of many aërial hyphae. The growth was sparse but spread quickly; evidently the nitrogen of the inoculum could be made use of even when spread out quite thinly. The cells of these plates were very commonly bulbous at both ends.

## Discussion

The results of these experiments indicate that the organism attacks a great variety of substances. Duggar's conclusion that sugars fail to support it is shown to be erroneous; his culture solution was probably made too concentrated by the addition of sugars. The growth with xylose, dextrose, and maltose supports the opinion that the mycelium grows upon complex organic matter by reason of the production of sugars. It would be interesting to attempt a culture upon cellobiose, which is supposed to be produced by organisms which break down cellulose.

Cellulose supported only a little growth when spread out on silica gel.

There was more opportunity for the products of hydrolysis to diffuse away from the mycelium than in the preliminary paper base cultures, where the paper was packed in tightly. In a recent experiment the writer corroborated the earlier evidence of cellulose breakdown, by measuring the loss in weight of small pieces of filter paper attacked by the mycelium. The loss averaged about 4 percent, and the mycelium was necessarily weighed with the paper. The fibers were swollen and frayed at the ends. In spite of this it seems most likely that the cellulose in a material such as manure is broken down more slowly than some other constituents.

The surprising growth on peach gum and commercial pectin adds two more classes of materials to the list of available foods. The results with the hemicelluloses tested are not very conclusive. The lignin-bearing materials produced the fastest growing cultures of the series. It is important that this mushroom seems to belong with the lignin-destroying organisms, although this must not be considered established by this very limited study. According to Waksman (12) the decomposition of organic matter is usually accompanied by the accumulation of lignin or substances of the same nature, due to the inability of most organisms to attack it. The final residue of such decomposition is of a humus or peaty nature. In support of the theory that *Agaricus campestris* can break down and utilize lignin it may be noted that when the spent manure from mushroom beds is applied to the soil it soon disappears and does not seem to add to the humus of the soil. It would seem that lignin had not accumulated in the mushroom bed.

The growth upon the proteins was not quite typical of the organic series. It was dense and not dendritic. Strands did not appear in most cases until the medium became exhausted. Whether considered from the point of view of organic or of nitrogenous nutrition, it appears that the proteins are very suitable nutrients. From the researches of Waksman (12) we learn that a considerable change of nitrogen from urea and ammonium salts to protein takes place when manure is composted. This process would probably favor the nutritional preferences of the mushroom, and probably takes place in the composting of manure for mushroom growing. The ammonium salts and amino-acids served well as nitrogen sources, as was to be expected, for proteins must be broken down into some of these compounds before the organism can utilize them.

The occurrence of strands in certain plates has no special significance with reference to nutrition, so far as the writer has been able to observe.

The appearance of crystals on the hyphae was noted in all the cultures. More crystals were produced in some than others; the pectin cultures were thickly covered with them. Hein (6) observed crystals which he stated were calcium oxalate. Since more crystals appeared on the aërial hyphae than on the others, and since some of these were soluble in water, it seems possible that some other organic salt or acid was also produced. The pH of the medium is invariably lowered by the spawn in commercial mushroom

production, finally arriving at a value of about 4.5. The same effect was produced in the cultures of Frear (5), with nutrient solutions on a paper base. This pH change may have a profound influence upon the further life of the mycelium; and it may possibly be prevented by chemical means with great benefit to the mycelium. The further study of this matter is being undertaken with hope of useful results.

It should now be possible to make silica gel and also larger scale cultures upon many materials, including composts and fractions prepared from them, and to correlate the results with analytical studies. There should result many improvements in the handling of manures for mushroom production, as well as better understanding of the principles involved. The microbiology of manures will most certainly be found of profound importance. After finding that the organism can obtain nutrients from so many classes of organic compounds, the writer sees no good reason why many other materials can not replace manure in mushroom production.

## Summary

1. The mycelium of the "Snow-White" variety of *Agaricus campestris* does not tolerate a medium of nutrient salts and soluble organic substances, in which the total concentration is much above 0.2 *M*.

2. The mycelium is intensely aërobic. In fibrous media it will not tolerate the addition of water if aëration is cut off thereby. Mycelial strands develop profusely in the wetter media, and as their formation necessarily precedes fruiting, a connection between aëration changes, mycelial strands, and fruiting is suggested.

3. The spores of *Agaricus campestris* germinate more quickly in large masses than when separated. The germination stimulus developed in the spore mass appears to be the same as that by which the living mycelium hastens germination.

4. The mycelium was grown on silica gel plates containing materials representative of the main classes of substances in manure and decomposing organic matter. Growth was good with xylose, dextrose, maltose, peach gum, commercial pectin, wheat bran, wheat straw, *Liriodendron* wood, and commercial granulated peat moss; and also on a sample of wood from which nearly all but lignin had been removed by wood-rotting fungi. Several proteins were excellent sources of nitrogen and organic food. Cellulose failed to support vigorous growth. The organism probably can make use of nearly all the substances in manure including lignin. Cellulose, however, would probably not be among the first attacked.

5. The characteristic mycelial growth is dendritic with strand formation. On the proteins the dense hyphal growth is uniform in outline and few strands are produced.

The guidance and assistance of Dr. Rodney H. True throughout the progress of the work is acknowledged with the greatest pleasure. The

writer has also been fortunate in receiving most useful suggestions, as well as samples of some of the nutrients tested, from Dr. Selman A. Waksman.

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University of Pennsylvania

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